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RESEARCH

Laboratory Evaluation of Acute Toxicity of the Essential Oil of *Allium tuberosum* Leaves and Its Selected Major Constituents Against *Apolygus lucorum* (Hemiptera: Miridae)

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ABSTRACT. The aim of this research was to evaluate acute toxicity of the essential oil of leaves of Chinese chives, *Allium tuberosum* Rottler ex Spreng (Asparagales: Alliaceae) and its major constituents against *Apolygus lucorum* Meyer-Dür (Hemiptera: Miridae). The essential oil of *A. tuberosum* leaves was obtained by hydrodistillation and analyzed by gas chromatography and gas chromatography-mass spectrometry. The major constituents of the oil were sulfur-containing compounds, including allyl methyl trisulfide (36.24%), diallyl disulfide (27.26%), diallyl trisulfide (18.68%), and dimethyl trisulfide (9.23%). The essential oil of *A. tuberosum* leaves exhibited acute toxicity against *Ap. lucorum* with an LD_{50} value of 20.03 μg per adult. Among the main compounds, diallyl trisulfide ($LD_{50} = 10.13$ μg per adult) showed stronger acute toxicity than allyl methyl trisulfide ($LD_{50} = 21.10$ μg per adult) and dimethyl trisulfide ($LD_{50} = 21.65$ μg per adult). The LD_{50} value of diallyl disulfide against *Ap. lucorum* was 28.10 μg per adult. The results indicated that the essential oil of *A. tuberosum* and its major constituents may have a potential to be developed as botanical insecticides against *Ap. lucorum.*

Key Words: acute toxicity, essential oil, *Allium tuberosum*, *Apolygus lucorum*

During the last decade, the population density of *Apolygus lucorum* Meyer-Dür (Hemiptera: Miridae) has increased dramatically due to the massive cultivating of the genetically modified cotton (Bt cotton) and the control of the most destructive insect, the cotton bollworm, *Helicoberpa armigera* Hübner (Lu et al. 2010). The characteristics of *Ap. lucorum*, e.g., the environmental adaptability, high population growth rate, and strong spreading ability, cause them easily attaining outbreak density (Lu et al. 2007), and the mirid bugs has become the major cotton pest in the Yellow river and Yangtze river basin, China. In 2008, the cotton fields invaded by *Ap. lucorum* covered an area of 3.9 million hm^2, resulting in a loss of 5 million kg of cotton (Lu et al. 2010). Currently, the control of *Ap. lucorum* mainly depends on the usage of synthetic insecticides, such as fipronil, lambda-cyhalothrin, and carbosulfan in China. However, repeated usage of these synthetic insecticides has caused several problems, such as disruption of the natural biological control systems, and sometimes resulted in the widespread development of resistance and undesirable effects on nontarget organisms (Isman 2004). Therefore, developing alternative strategy of using eco-friendly products is urgently needed.

Botanical insecticides including essential oils from the perspective above provide new patterns of insecticides that have the advantage of avoiding the risk of chemical insecticides in terms of low mammalian toxicity, rapid degradation, and local availability (Isman 2006, 2008). Several essential oils showed insecticidal activity against Pentatomidae insects with sucking mouth-part. For example, 13 essential oils, especially caraway and clove bud oils, could effectively repel the bean bug, *Riptortus clavatus* (Yang et al. 2009), while the essential oils of *Origanun vulgare* (oregano) and *Thymus vulgaris* (thyme) produced contact and fumigant toxicity against nymphs and adults of the southern green stink bug, *Nezara viridula* (Werdin Gonzalez et al. 2011). Moreover, Zhang et al. (2014) tested eight kinds of essential oils (clove, lemongrass, spearmint oil, ylang-ylang oil, wintergreen oil, geranium oil, pennyroyal oil, and rosemary) and indicated that all of them showed repellency against both nymphs and adults of the brown marmorated stink bug, *Halyomorpha halys*. During our mass screening program for new agrochemicals from Chinese medicinal herbs, the essential oil of *Allium tuberosum* Rottler ex Spreng (Asparagales: Alliaceae) leaves showed insecticidal activity against *Ap. lucorum* adults.

Chinese chives, *A. tuberosum,* is a perennial plant cultivated in many countries in Asia and its aerial parts are one of the common edible green vegetables for the Chinese. *A. tuberosum* showed obviously odor analogous to the smell of the garlic and other *Allium* plants. The smelly odor was caused by the sulfur-containing compounds (Block 2013). In the previous researches, sulfur-containing compounds (such as allyl methyl disulfide, diallyl disulfide, allyl methyl trisulfide, dimethyl disulfide, allyl methyl sulfide, and diallyl sulfide), aliphatic aldehydes, ketones, and aliphatic alkanes have been identified from Chinese chive's leaves and seeds (Lopes et al. 1997, Hu et al. 2013). Chinese chives oil was reported to show significantly repel and contact toxicity against the adults of Asian citrus psyllid, *Diaphorina citri* (Mann et al. 2011, 2012) and also demonstrated antifungal activity (Rattanachaikunsopon and Phumkhachorn 2009, Kocevski et al. 2013). However, there is no information on insecticidal activity of the Chinese chives essential oil and its constituent compounds against *Ap. lucorum* after a literature survey. Thus, in this research, we tried to investigate acute toxicity of the essential oil of *A. tuberosum* and its major concentrations against *Ap. lucorum*.

Materials and Methods

Plant and Extractions. The leaves of *A. tuberosum* (50 kg) were purchased in April 2014 from Beijing Chaoshifa Chain Store Co. Ltd. in Haidian District, Beijing, China. The plant material was identified by Dr. Liu QR (College of Life Sciences, Beijing Normal University, Beijing 100875, China), and a voucher specimen (CFP-Jiucai-Haidian-2014-06) was deposited at the Department of Entomology, China Agricultural University, Beijing, China. Each portion of fresh leaves (500 g) was managed into juice by a fruit blender. The essential oil of the fresh leaves of *A. tuberosum* was extracted with *n*-hexane by hydrodistillation by means of a Clevenger-type apparatus for 6 h. The solvent was evaporated using a BUCHI Rotavapor R-124 vacuum rotary evaporator (BUCHI, www.buchi.com) at 40°C. The oil sample was dried over anhydrous sodium sulfate and kept in airtight containers in a refrigerator at 4°C prior to analysis. Dimethyl trisulfide (98%), diallyl trisulfide (98%), and diallyl disulfide (85%) were purchased from Aladdin-Reagent Company (Shanghai, China). Allyl methyl trisulfide (98%) was purchased from Chengdu XiYa Chemical Technology Co., Ltd. (Chengdu, China). Positive control, the garlic oil (diallyl trisulfide [50.4%], diallyl disulfide [25.3%], diallyl sulfide [6.3%]) (Zhao et al. 2013) was purchased from Jinyuan Xingke Technology Limited (Daxing District, Beijing, China).

The Analysis of *A. tuberosum's* Oil by Gas Chromatography and Mass Spectrometry. The analysis of the essential oils was performed on gas chromatography (GC)-flame ionization detection and GC-mass spectrometry using a Agilent 6890N gas chromatograph coupled to an Agilent 5973N mass selective detector (www.agilent.com) equipped with capillary column with HP-5MS (30 m by 0.25 mm, df = 0.25 μm). The carrier gas was He and was used at 1.0 ml min^{-1} flow rate. The oven temperature program was as follows: held at 60°C for 1 min and ramped at 10°C·min^{-1} to 180°C and held there for 1 min, and then ramped at 20°C min^{-1} to 280°C where it was held there for 15 min. The chromatograph was equipped with a split or splitless injector used in the split mode. The temperature of the injector maintained at 270°C, then injected the samples (1 μl, diluted to 1% with acetone) with a 1:10 split ratio. Spectra were scanned at 2 scans·s^{-1} from 20 to 550 *m/z*. Identification of components was assigned by matching their mass spectra with those of dependable compounds available in our laboratories or with those from other literatures. Under the same operating conditions, the retention indices were verified in relation to a homologous series of *n*-alkanes (C_8–C_{24}). Further identification was made with comparison of their mass spectra with those stored in Wiley 275 libraries (Wiley, New York, NY) and NIST 05 (Standard Reference Data, Gaithersburg, MD), otherwise with mass spectra from literature (Adams 2007). The component concentration was obtained by semi-quantification by peak area integration from GC-flame ionization detection peaks and by applying the correction factors.

Insect Cultures and Rearing Conditions. The colony of *Ap. lucorum* was established on September 2012 at the Department of Entomology, China Agricultural University, Beijing, China, from individuals captured from an experimental field in Shangzhuang, Beijing, China. The colonies for bioassay were kept in 20 by 10 by 6 cm transparent plastic rearing containers at 28–30°C, 55–65% relative humidity (RH), and a photoperiod of 14:10 (L:D) h in culturing chambers. In each container, fresh corn (*Zea mays*) kernels were provided as a food source and oviposition material. The corn kernels with eggs were collected every 2 d and transferred into a new container until nymphs emerged, then we put them into a new container with corns as food which were refreshed every two days. Usually, the nymphs take 17–19 d grow to adults, and the new adults within 24 h were used in the experiments.

Bioassays. Preliminary experiments were carried out to set up appropriate experimental concentrations according to the methods (Zhang et al. 2009). Six concentrations of each test essential oil or compounds were prepared by dissolving the test liquids in acetone, namely, the essential oil of *A. tuberosum* and the essential oil of *Allium sativum* (0.48–5.00%), allyl methyl trisulfide (1.34–5.00%), diallyl disulfide (0.69–13.00%), diallyl trisulfide (0.58–2.60%), and dimethyl trisulfide (2.00–5.00%). Without any precipitation or phase partitioning upon dilution, every essential oil and compound was readily soluble in acetone. Therefore, only acetone solution was as negative control. Because the oil of *A. sativum* had been developed insecticides and effectively control *Ap. lucorum* in our preliminary experiments, it was used as the positive control in this study. The mirid bugs were sedated on the icebag for about 3 min to facilitate oil or compound application and transferred into a 90 mm Petri dish (10 insects per dish). The ice-anesthetization had no effect on the survival of *Ap. lucorum* because more than 95%insects survived in control treatments; 0.8 μl of every serial dilution of essential oil or compound or only acetone treatment as control was applied to the dorsal thorax of *Ap. lucorum* adults from lower to higher concentration by using a microsyringe. Five replications were used in all the treatments and each replications included 10 insects. The experiments were conducted in a culturing chamber [28-30°C, 55-65% RH, and a photoperiod of 14:10 (L:D) h]. The mortalities of *Ap. lucorum* adults were defined as the insects unable to crawl when stimulated with a hairbrush and was recorded after 24 h. The results of six essential oils or compounds were subjected to probit analysis to verify LD_{50} values and their 95% confidence intervals with the PoloPlus Version 2.0 (Kabir et al. 1996).

Results

The yield of essential oil of *A. tuberosum* was 0.005% (v/w based on fresh weight), while its density was determined to be 1.018 g/ml. A total of 20 components (19 of them were sulfur-containing compounds) from the essential oil of *A. tuberosum* were identified, accounting for 97.95% of the total oil. The principal constituents of A. tuberosum essential oil were allyl methyl trisulfide (36.24%), diallyl disulfide (27.26%), diallyl trisulfide (18.68%), and dimethyl trisulfide (9.23%) (Table 1).

The essential oil of *A. tuberosum* exhibited acute toxicity against *Ap. lucorum* with an LD_{50} value of 20.03 μg per adult (Table 2). The constituent, diallyl trisulfide possessed acute toxicity against *Ap. lucorum* with an LD_{50} value of 10.13 μg per adult, while allyl methyl trisulfide, diallyl disulfide, and dimethyl trisulfide had LD_{50} values of 21.10 μg per adult, 28.10 μg per adult, and 21.65 μg per adult, respectively (Table 2).

Discussion

This study is the first report on the acute contact toxicity of essential oil from *A. tuberosum* against *Ap. lucorum*, a major cotton pest in China now. Our present research found that allyl methyl trisulfide, diallyl disulfide, diallyl trisulfide, and dimethyl trisulfide were the major constituents in the essential oil of *A. tuberosum* leaves. Although the volatile constituents of the essential oil of *A. tuberosum* were mainly sulfur-containing compounds, the relative proportions of these sulfides might vary according to different collection places, different extraction parts, or both. For instance, the essential oil of *A. tuberosum* leaves cultivated in Brazil contained allyl methyl disulfide (25.9%), diallyl disulfide (22.5%), allyl methyl trisulfide (9.4%), dimethyl disulfide (5.3%),

Table 1. Chemical constituents of the essential oil of *A. tuberosum* leaves

Peak no.	Compound	RI	Content (%)
1	Allyl methyl sulfide	702	0.26
2	Dimethyl disulfide[a]	760	0.39
3	Diallyl sulfide[a]	848	0.13
4	2-Hexenal	853	0.69
5	2,4-Dimethyl thiophene	885	0.43
6	Allyl isothiocyanate	890	0.18
7	Allyl Methyl disulfide[a]	915	0.07
8	Methyl propyl disulfide	950	1.09
9	Dimethyl trisulfide[a]	975	9.23
10	1,3-Dithiane	1,027	0.19
11	Diallyl disulfide[a]	1,077	27.26
12	Dipropyl disulfide	1,098	0.12
13	Allyl methyl trisulfide[a]	1,134	36.24
14	Methyl propyl trisulfide	1,168	2.08
15	Dimethyl tetrasulfide	1,224	0.42
16	Diallyl trisulfide[a]	1,296	18.68
17	Diallyl thiosulfinate	1,325	0.12
18	Dipropyl trisulfide	1,328	0.04
19	Allyl methyl tetrasulfide	1,386	0.07
20	Diallyl tetrasulfide	1,540	0.26
	sulfur-containing compounds		97.26
	others		0.69
	Total identified		97.95

RI, retention index, as determined on an HP-5ms column using the homologous series of *n*-hydrocarbons.

[a]Identification by coinjection of authentic compounds.

Table 2. Acute toxicity of the essential oil of *A. tuberosum leaves* and its major constituents against *Ap. lucorum* adults

Treatment	LD_{50} (μg/adult)	95% Fiducial limits	Degrees of freedom	Slope ± SD	Chi-square value
Allyl methyl trisulfide	21.1	19.40-22.86	28	4.74 ± 0.50	14.04
Diallyl disulfide	28.1	25.39-30.78	28	2.74 ± 0.26	13.58
Diallyl trisulfide	10.13	9.26-11.10	28	3.86 ± 0.38	12.31
Dimethyl trisulfide	21.65	20.49-22.89	28	6.99 ± 0.71	10.30
A. tuberosum	20.03	18.81-22.50	28	4.39 ± 0.45	12.36
A. sativum	13.36	12.62-14.34	28	2.80 ± 0.28	12.28

allyl methyl sulfide (5.0%), and diallyl sulfide (5.0%) (Lopes et al. 1997). However, when the leaves of *A. tuberosum* were collected in Havana, Cuba, the main components of their essential oil were methyl propyl trisulfide (9.9%), dimethyl disulfide (7.3%), dipropyl trisulfide (6.0%), dimethyl trisulfide (6.0%), methyl propyl disulfide (5.5%), and dipropyl disulfide (5.5%) (Pino et al. 2001). Moreover, there were differences among different parts of *A. tuberosum* in constituents when being analyzed, e.g., hexanal (15.8%), 2-pentyl furan (7.3%), methyl 2-propenyl disulfide (6.7%), methyl isopropyl disulfide (5.4%), diallyl disulfide (5.4%), and dimethyl tetrasulfide (5.2%) were major components in the essential oil of *A. tuberosum* seeds (Hu et al. 2013). Zhang et al. (2013) demonstrated higher contain of dimethyl disulfide and dimethyl trisulfide from the Chinese chives roots than those from the leaves. Thus, for practical use, it was necessary to standardize the concentrations of the components of the essential oil of *A. tuberosum* leaves.

The essential oil of *A. tuberosum* leaves showed less acute toxicity against the adults of *Ap. lurocum* than the garlic essential oil ($LD_{50} = 13.36$ μg per adult, Table 2). Garlic essential oil was chosen as a positive control because of its strong insecticidal activity, for example, fumigant activity against the Japanese termite, *Reticulitermes speratus* (Park and Shin 2005), larvicidal activity to *Ae. albopictus* (Tedeschi et al. 2011), contact toxicity against pear psyllid, *Cacopsylla chinensis* (Zhao et al. 2013). In fact, the essential oil and other extracts from *A. sativum* had been developed into a series of pest control insecticides for use against several pests, e.g., Garlic Barrier Ag (Garlic Barrier AG, Glendale, CA) and ENVIR epel (Cal Crop USA, Greeley, CO).

Our present bioassay showed that diallyl trisulfide had the strongest acute toxicity against *Ap. lucorum* adults among the four major compounds. Diallyl trisulfide exhibited almost twice as acute contact toxicity against *Ap. lucorum* as the essential oil of *A. tuberosum* leaves and even stronger than the positive control, the essential oil of *A. sativum* (no overlap in 95% fiducial limits (FL)). The mirid bug was less susceptible to both allyl methyl trisulfide and dimethyl trisulfide than to diallyl trisulfide. Diallyl disulfide was the weakest on the acute toxicity to *Ap. lucorum* adults (Table 2). Therefore, we suggested that the three main compounds dially trisulfide, allyl methyl trisulfide, and dimethyl trisulfide might be attribute to the acute toxicity of the essential oil of *A. tuberosum* leaves. In addition, diallyl trisulfide has been demonstrated to show insecticidal activity against *Tribolium castaneum* (Koul 2004) and *Sitophilus zeamais* (Huang et al. 2000) and also detected to have strong fumigant toxicity against *R. speratus* (Park and Shin 2005). Moreover, several minor constituents in the *A. tuberosum* essential oil might participate in acute toxicity against *Ap. lucorum*. Campbell et al. (2011) demonstrated that diallyl tetrasulfide also acquires repellency against the adults of yellow fever mosquito, *Ae. aegypti*. It was also reported that 1, 3-dithiane possessed insecticidal and acaricidal activity as well (Hideki et al. 1992). Additionally, dimethyl disulfide demonstrated repellency against *Ap. lucorum* adults (Pan et al. 2013).Therefore, it is necessary to pursue a further investigation on the insecticidal activity against *Ap. lucorum* of those minor constituent compounds and to figure out the existence of some kind of synergy among the constituent compounds of the essential oil of *A. tuberosum* leaves.

A. tuberosum, for long time were used as a traditional Chinese vegetable and medical herb, because they were cheap, environmental-friendly, and usually safe for human consumption. Furthermore, garlic has been utilized worldwide to treat many kinds of diseases, such as cough, toothache, hypertension, earache, and atherosclerosis (Rivlin 2001). However, it is necessary to do a further research of toxic effects on humans in the essential oil of *A. tuberosum* and its major constituents, and the actual impact on crops and natural enemies in crop fields should also be under consideration. In this study, the bioassay was conducted in the laboratory, but it is necessary to do a further field study to improve the results of laboratory tests and make a comprehensive assessment of the insecticidal ability of the essential oil of *A. tuberosum* and its major constituents.

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